Melting of local ordered structures in yeast 5S ribosomal RNA in aqueous salts

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ABSTRACT

Equilibrium and kinetics of thermal melting of yeast 5S ribosomal RNA in aqueous NaCl with or without Mg^{2+} were investigated by differential thermal melting and temperature jump methods. Two peaks (1 and 2) and a shoulder were observed in each of melting curves at ionic strength $I=0.002-0.5$ and linearity between each of melting temperatures T_m and T_m^0 and $\log I$ was found at $I=0.01-0.5$ in the Mg^{2+} -free solution. The local structures were found to be stabilized considerably by Mg^{2+} . The temperature jump measurements gave the kinetic melting curve of the structure 1 at $I=0.03$ without Mg^{2+} or with $0.5 \text{ mM } Mg^{2+}$. The kinetic T_m coincided well with the corresponding static T_m . For the structure 1, various parameters were calculated from the kinetic data, which indicated a double helical character of the structure 1. In terms of the values of T_m , G-C content, and enthalpy change of the transition of the structure 1 or 2, appropriateness of each of the secondary structure models of eukaryotic 5S RNA proposed previously was discussed.

INTRODUCTION

5S RNA is a structural component of every prokaryotic and eukaryotic ribosome (1,2). Prokaryotic 5S RNA is implicated in binding of all tRNAs to the ribosome, while function of eukaryotic 5S RNA has remained uncertain. Important properties of prokaryotic 5S RNA and eukaryotic 5S RNA are different, while prokaryotic 5S RNA and eukaryotic 5.8S RNA show similarity (3-5). Many previous studies have characterized conformation of prokaryotic 5S RNA and they have suggested a high degree of base pairing and a largely double helical configuration (6-8), as in tRNAs. Models of secondary and tertiary structures in prokaryotic 5S RNA have been introduced from results of optical absorption, Raman spectroscopy, enzyme digestion, chemical modification and theoretical calculation studies (1,5,6,9,10). High resolution NMR spectra were found to be important to make the models (11,12). Moreover, some dynamic characters of E.coli 5S RNA were clarified by means of temperature jump method (13).

For eukaryotic 5S RNA, the conformational studies have been attempted. Optical (14-16), Raman (17) and chemical methods (9,18) have given equilibrium properties of eukaryotic 5S RNA, in which a high degree of base pairing and a

largely double helical configuration have also been expected to exist. However, much of its structure has yet remained uncertain. The monophasic ultraviolet melting profile of yeast 5S RNA has been obtained by some authors (14, 15), but others have shown the biphasic melting curve of it (3,19). The first transition in the thermal melting process was suggested, by Stulz et al. (16), to be concerned with change in the tertiary structure in yeast 5S RNA, which has not been pointed out to be a process involved in the transition according to Luoma et al. (19), however. Number of the base-pair in yeast 5S RNA determined from NMR spectra by Luoma et al. (19) did not coincide with that determined previously from NMR data by Wong et al. (15). Moreover, their NMR data gave only the equilibrium properties such as the number of base-pair in the RNA. Few dynamic character of eukaryotic 5S RNA has yet been found, except in a work by Maruyama and Sugai (20), who characterized the kinetic behaviors of folding of yeast 5S RNA by Mg^{2+} . The kinetic studies on the thermal unfolding of eukaryotic 5S RNA are necessary to discuss the conformation of the RNA.

In this report, equilibrium and kinetics of reversible thermal unfolding of yeast 5S RNA in aqueous NaCl with or without Mg^{2+} are described by means of differential thermal melting and temperature jump methods. The results were discussed in terms of the secondary structure models of eukaryotic 5S RNA proposed previously (5,9,18,19,21).

MATERIALS AND METHODS

Yeast 5S RNA was prepared from yeast (*Saccharomyces cerevisiae*) cells by Holley's method (22). The 5S RNA was separated from other small RNAs (tRNAs and 5.8S RNA) through two chromatographic procedures: first, ion exchange chromatography on DEAE-Sephadex A-25 in 1M phosphate with a concentration gradient of KCl, then gel filtration on Sephacryl S-200 in 0.02 M Tris(pH 7.5) and 0.2 M KCl. The monodispersed fraction on polyacrylamide gel was sufficiently dialysed against deionized water and it was filtrated through a membrane filter with a pore size of 0.45 μ m prior to use. The RNA concentration was determined spectrophotometrically as in the previous paper (14,20).

To eliminate the bound Mg^{2+} from our RNA, the aqueous solution of the RNA was dialysed against 100 mM Tris and 30 mM EDTA at pH 7 for a day at room temperature (a), for half an hour at 60°C and then cools down to 25°C slowly (b), and for an hour at 60°C and then cools down to 4°C rapidly (c). The melting of the 5S RNA obtained by the procedure (a) or (b) was not completely reversible, and that of the RNA by the procedure (c) was irreversible. Therefore, we did not use the RNA from the procedure (a), (b) or (c).

Other chemicals were of reagent grade (Wako Pure Chem. Ind., Ltd., and Nakarai Chem., Ltd.).

Differential thermal melting was obtained at both 260 and 280 nm in a spectrophotometer (Union SM-401), as described previously (23). Heating was performed at a rate of $\sim 0.5^\circ\text{C min}^{-1}$ from 10 to 95°C . An optical cell of path length 0.5 cm was used.

The temperature jump measurements were carried out in a rapid reaction analyser (Union RA-410), as described previously (23,24). A temperature jump size of 4.5°C was used in this study. Rapid change in transmittance at 260 nm caused by the temperature jump was memorized and accumulated 20-30 times in a microcomputer (Kawasaki Electronica TM-410), and the data were analysed by another computer (Sord M-222) to get the kinetic parameters.

RESULTS

Thermal Melting

The differential thermal melting curve of yeast 5S RNA in a citrate buffer with NaCl and EDTA (pH=7.0 and ionic strength $I=0.1$) recorded at 260 nm is shown in Fig. 1, in which two peaks (1 and 2) are clearly observed. Each peak corresponds to melting temperature T_m of an ordered structure. Change in T_m between the structures 1 and 2 is scarce. Values of dA_{280}/dA_{260} , which ref-

Figure 1. Differential thermal melting curves in citrate buffers with NaCl and EDTA ($[\text{NaCl}]:[\text{citrate}]:[\text{EDTA}]=970:10:1$) at pH=7.0 and $I=0.10$ (a), 0.50 (b). (c) A_{260} vs. temperature at $I=0.10$. $[\text{RNA}]=1.5 \cdot 10^{-6}$ monomole/l.

lects the G-C content (25), of the structures 1 and 2 were also very similar ($[G-C]=56\pm 2\%$). Another local structure 3, which melts at 35°C and may be concerned with the tertiary structure, should be noted to be found in the curve as a shoulder.

The ionic strength dependence of T_m was determined for the two structures 1 and 2. The T_m^2 could be estimated only at $[\text{Na}^+] < 0.5 \text{ M}$, because location of the peak 2 was obscure at $I=0.5$ (as shown in Fig. 1). Plots of T_m vs. $\log[\text{Na}^+]$ shown in Fig. 2 indicate linearity of each plot at $0.5 > I > 0.01$. Values of the slope, $dT_m/d\log[\text{Na}^+]$, and the T_m 's at $[\text{Na}^+]=1 \text{ M}$ are shown in Tables 1 and 2, and they are compared with the results for other nucleic acids.

The structures 1, 2, and 3 are stabilized by Mg^{2+} , and melting of the structures 2 and 3 can not be separated at $[\text{Mg}^{2+}] > 10 \text{ mM}$ in the cacodylate buffer at $\text{pH}=7.0$ and $I=0.1$, as shown in Fig. 3(A). Plots of T_m vs. $[\text{Mg}^{2+}]$ are shown for the two structures 1 and 2 at $I=0.1$ and $\text{pH}=7.0$ in Fig. 3(B).

Temperature Jump Measurements

As shown in Fig. 4, at least three kinetic processes could be recognized from change in transmittance at 260 nm after temperature jump to final temperature T_f in this study: very fast (in a time range less than $100 \mu\text{s}$), intermediate (in a range of a few msec), and slow (in a range longer than 100 msec)

Figure 2. Dependence of T_m^1 and T_m^2 on $\log[\text{Na}^+]$ in the citrate buffers shown in Fig. 1. Triangles represent T_m at $[\text{Na}^+]=1 \text{ M}$ estimated by extrapolation.

Table 1. Values of $dT_m/d\log[Na^+]$ of various RNAs.

RNA	$dT_m^1/d\log[Na^+]$	$dT_m^2/d\log[Na^+]$	$dT_m^3/d\log[Na^+]$	Reference
Yeast 5S	17	22	-	this study
Yeast 5.8S	19	20	-	(23)
E.coli tRNA ^{fMet}	20	23	13	(27)
E.coli tRNA ^{Tyr}	27	8	15	(27)
Double-strand Reovirus RNA	14	14	14	(28)
PolyA-PolyU	20			(29)
PolyG-PolyC	9			(28)

ones. The fast process, which may be due to unstacking of base, was observed by the jumps to any T_f and it could not be studied here, because of rapidness. As T_f approached to T_m^2 , the intermediate process became remarkable. Also, the slow one became a main course of kinetics, as T_f approached to T_m^1 , and then it may be concerned with melting of the structure 1. The kinetics of this pro-

Table 2. Parameters of the local ordered structures in yeast 5S and 5.8S (23) RNAs

Local Structure in RNA	$[Mg^{2+}]_{add}$ (mM)	Method	I (M)	T (°C)	[G-C] (%)	ΔH	E_f^* (Kcal/mol)	E_r^*
1 in 5S	0	static	1.0	73	56±2			
	0	static	0.03	48				
	0	kinetic	0.03	47		43±5	21±1	-23±1
	0.5	static	0.03	58				
	0.5	kinetic	0.03	56		53±6	26±1	-29±1
2 in 5S	0	static	1.0	67	56±2			
	0	static	0.03	34				
1 in 5.8S	0	static	1.0	103	91			
	0	static	0.03	78		86		
	0	kinetic	0.03	75		94±5	63±1	-29±1
2 in 5.8S	0	static	1.0	65	56			
	0	kinetic	0.03	31		70±2	45±3	-31±4

E^* expresses activation energy.

Figure 3(A). Differential thermal melting curve in a cacodylate buffer of 1 mM with NaCl, EDTA of 0.1 mM and MgCl_2 of 0.5 mM at pH=7.0 and $I=0.1$.

Figure 3(B). Dependence of T_m^1 and T_m^2 on concentration of added Mg^{2+} in the cacodylate buffer of 1 mM shown in Fig. 3(A) at pH=7.0 and $I=0.1$.

cess was in details investigated in this study.

The calculated kinetic amplitude of the slow process at $[\text{Na}^+]=30$ mM is shown in Fig. 5(A) and it is assumed to fit to a Gaussian form. The kinetic melting temperature agrees well with the T_m^1 in the static melting curve at 30 mM Na^+ in the citrate buffer. The apparent rate constant k_{app} of the process

Figure 4: Time course of transmittance at 260 nm of the RNA in the citrate buffer of 1 mM with NaCl and 0.1 mM EDTA at pH=7.0 and $I=0.1$ after the jump to 49°C. The insert represents the semi-log plot of absorbance vs. time.

Fig. 5(A). The kinetic melting curves of the RNA in the citrate buffer (0.3 mM) with NaCl and 0.03 mM EDTA at pH=7.0 and $I=0.03$ (a), and in the cacodylate buffer (0.3 mM) with NaCl, 0.03 mM EDTA and 0.5 mM $MgCl_2$ at pH=7.0 and $I=0.03$ (b).

Fig. 5(B). Temperature dependence of the apparent rate constant of the slow process in the citrate buffer shown in Fig. 5(A) at pH=7.0 and $I=0.03$.

is plotted against reciprocal of temperature in Fig. 5(B). A minimum of it is found at the kinetic T_m . Also, relaxation rate seems to be reasonable for a double helix to coil transition compared with those observed in several tRNAs (26) and yeast 5.8 S RNA (23).

The forward and backward rate constants, k_f and k_r , of the transition were calculated by the same method as in the previous kinetic study of yeast 5.8S RNA (23). The equilibrium constant of the transition was calculated from the data in Fig. 5(A). Both k_f and k_r are plotted against reciprocal of temperature in Fig. 6(A) and activation energies were calculated as shown in Table 2.

The kinetic melting curve of yeast 5S RNA in aqueous cacodylate with 0.5 mM Mg^{2+} at $I=0.03$ is shown in Fig. 5(A), in which stabilization of the structure 1 by Mg^{2+} is seen. Change in the kinetic T_m^1 by 0.5 mM Mg^{2+} coincides with that in the static profile. Both the rate constants, k_f and k_r , in the solution with 0.5 mM Mg^{2+} are shown in Fig. 6(B). The T_m^1 , where $k_f=k_r$, agrees with the kinetic T_m^1 shown in Fig. 5(A). The activation energies from Fig. 6(B) are also shown in Table 2.

Fig. 6(A). Temperature dependence of k_f and k_r in the citrate buffer of 0.3 mM at pH=7.0 and I=0.03.

Fig. 6(B). Temperature dependence of k_f and k_r in the cacodylate buffer with 0.5 mM Mg^{2+} at pH=7.0 and I=0.03.

DISCUSSION

Maruyama et al. have previously described the thermal melting of yeast 5S RNA (14), which was prepared by the same method as in this study, and the melting was reported to be in a monophasic type at low I (=0.016). They did not use the differential technique. Luoma et al. (17) indicated the biphasic plots of absorbance at 260 or 280 nm vs. temperature at I=0.1. On the other hand, Willick et al. (3) have recently used the differential technique, by which they have shown existence of the two T_m 's, which coincide well with ours in this study. The T_m 's of the structures 1 and 2 are linearly dependent on log I at I=0.01-0.5. Our equilibrium data mean that 1) at least three local structures melt separately in aqueous Na^+ at pH=7.0 and I=0.01-0.5 between 10 and 95°C, 2) the two local structures 1 and 2 have similar stabilities and similar [G-C] values, and 3) contribution of the third structure to absorbance at 260 or 280 nm is not so remarkable. The T_m at I=0.016 reported previously by Maruyama et al. (14) coincides well with our T_m^1 at I=0.016. The apparent T_m for Mg^{2+} -deficient yeast 5S RNA at I=0.1 in the work by Luoma et al. (17) is between T_m^1 and T_m^2 at I=0.1 in this study. Also, change in the apparent T_m

by 10 mM Mg^{2+} at $I=0.1$ in their work is very similar to the change by 10 mM Mg^{2+} in this study.

At $I<0.01$, each of the $dT_m/d\log[Na^+]$ values is different from the corresponding ones at $I>0.01$. A similar change is found in a plot of T_m vs. $\log I$ for E.coli tRNA^{fMet} in aqueous Na^+ . The 5S RNA may change the conformation at low $I<0.01$.

Anyway, it may be concluded that the native yeast 5S RNA has at least three local structures being able to melt between 10 and 95°C at physiological I and they are stabilized by Mg^{2+} . It could not be determined in this study whether such stabilization of each structure is due to binding to specific site(s) or not.

The kinetic results in this study mean that the local structure 1 may be in a double helical type at $T<T_m^1$. Contribution of the structure 1 in the static differential melting curve could not be estimated, because the best-fitted curve to the melting curve could not be obtained by calculation with a microcomputer under an assumption of sum of two or three Gaussian forms. However, the parameters of the structure 1 could be estimated from the kinetic melting curve shown in Fig. 5(A), as shown for yeast 5.8S RNA (23). Scarce dependence of enthalpy change of the transition, ΔH , on I in its range between 0.03 and 1.0 may be assumed, as for the local structures in yeast 5.8S RNA (23). In Table 2, ΔH for the local structure 1 in yeast 5S RNA is shown at $I=0.03$. Activation energy for formation of the structure 1 resulted in a negative value, which indicates the nucleation proceeds for folding of double helical structure 1 in the RNA, as shown for double helical structures in tRNAs (30-32) and yeast 5.8S RNA (23). Values of ΔH and activation energies of the local structure 1 are reasonable for a double helix to coil transition.

On the other hand, for the structure 2, the kinetic melting curve could not exactly be estimated here, because of considerable rapidness. Therefore, the parameters of melting of the structure 2 can not be shown, however it is concluded to melt around 67°C at $I=1.0$.

We tried to calculate stability of each helix in the proposed secondary structure models of yeast 5S RNA by use of the parameters reported by Gralla and Crothers (33). The models used for calculation are shown schematically in Fig. 7. The transition parameters of a helical region in a model were firstly estimated, as if it was in an independent hairpin helix. An example of the hairpin helix (I) in the cloverleaf model is shown by a dotted curve in Fig. 7. The free energy of large loop was calculated by a method used for study of the tRNA model (34,35). Secondary, the parameters were corrected

Figure 7. The secondary structure models of eukaryotic 5S RNA by (a) Vigne-Jordan, (b) Luoma-Marshall, (c) Hori-Osawa, (d) De Wachter, and (e) Nishikawa-Takemura.

with the model, in which more unstable helices than the remarked helix melt. Vigne-Jordan model (18) has only one helical arm being able to melt between 50 and 80°C at $I=1.0$. Two helical arms in the cloverleaf model by Luoma and Marshall (17,19) can melt at 50–60°C at $I=1.0$, however others can not melt between 50 and 80°C. At $I=1.0$, two arms in Hori-Osawa model (5) melt at 50–90°C. Also, three arms (II, IV, V) in the De Wachter model (21) can open at 75, 71 and 68°C, respectively, at $I=1.0$. If one assumes that the arm IV and V melt independently, their melting may be expressed apparently by one peak in the static differential melting curve. Values of other parameters, ΔH and $[G-C]$, of the arms in De Wachter model are consistent with our data. De Wachter model may be appropriate for explanation of the equilibrium and kinetic melting data of the 5S RNA in this study. Lastly, at 50–90°C, three arms in Nishikawa-Takemura model (9) can melt at $I=1.0$. In terms of T_m , De Wachter model seems to be the most plausible one for some limited regions of yeast 5S RNA so far among the proposed five models of secondary structure in eukaryotic 5S RNA, however the cloverleaf and Hori-Osawa models are not so handy to explain our results.

The kinetic investigations of unfolding of the structures 2 and 3 in yeast 5S RNA are desirable to be attempted in future. Also, the equilibrium and kinetic studies on thermal melting of other eukaryotic 5S RNA should be achieved.

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